A FEASIBILITY INVESTIGATION OF THE POTENTIAL USE OF THE "CHARA PROCESS" AND INDIGENOUS BIOTA AS PASSIVE POLISHING AGENTS FOR CYANIDE IN WASTE LIQUORS AT ARVIDA

By

M. Kalin

For

D.O. Johnson
Alcan International Ltd.
Kingston Laboratories
Kingston, Ontario

April 8, 1985

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

1.0 SUMMARY

1.1 PURPOSE: Study the feasibility of utilizing an indigenous algal-bacterial flora and/or introduced Chara as passive polishing agents for removing cyanides from Arvida waste liquors.

1.2 SCOPE:

1.2.1 Conduct preliminary experiments to (1) determine the ability of Chara to survive in Arvida waste liquors and (2) test the potential of Chara to adsorb cyanides from waste liquors.

1.2.2 Collect and identify aquatic algal biota indigenous to the Arvida waste streams and grow the major indigenous taxa in the laboratory.

1.2.3 Conduct prelimfnary and simple experiments to determine whether the biota from Arvida can play a role in cyanide removal from waste streams.

1.3 CONCLUSIONS:

1.3.1 Chara alobularis, the only species available to be tested, survived in Arvida B and C stream effluents and some growth was recorded. Dead Chara showed a potential as a ferrocyanide bioadsorbant. Given the sodium-laden waste liquors, saline Chara species would appear more suitable for use in these waste streams.

1.3.2 The major aquatic algal taxa identified in seepages from the red mud pond and in waste stream C and B were blue-green algae, Phormidium and Oscillatoria. These algal-bacterial assemblages were isolated from all sites investigated. Presently 19 cultures are maintained and can be grown in the laboratory.

1.3.3 Preliminary and simple experiments with cultured Phormidum-Oscillatoria-bacteria assemblages did not conclusively demonstrate CN^- degradation.

The literature indicates that cyanide degradation by "Phormidium-Oscillatoria- bacteria assemblages" may be expected. No evidence was provided on the presence of significant concentrations of nitrate or ammonia in waste liquors effluents. An appropriate laboratory procedure has to 'mimic' the in-situ field conditions relative to CN^- as the sole nitrogen source.

Therefore, given the understanding gained during the feasibility study, the "passive polishing" potential of the indigenous Phormidium-Oscillatoria-bacteria populations is inferred from their presence in an aquatic habitat where cyanides may be the major nitrogen source for this extensive growth.

1.4 RECOMMENDATIONS:

1.4.1 "The Chara process"

A thorough assessment of the potential of several Chara species to grow at different locations, effluent streams and ponds is recommended.

Laboratory studies to determine the $Fe(CN)_6^{4-}$ adsorption ability of dead Chara spp. are indicated, in view of specific applications for the potlining leachate problem and emergency treatment measures.

1.4.2 Indigenous algae

Of primary importance are definitive laboratory experiments, which will demonstrate conclusively the degradation of cyanide by the indigenous "Phormidium-Oscillatoria-bacteria assemblages".

It is recommended that these studies are accompanied by laboratory growth studies with "Phormidium-Oscillatoria-bacteria assemblages" to determine their ability to utilize cyanide nitrogen for growth.

Field studies should be carried out at regular intervals during the growing season to determine the population dynamics of the algae in the waste streams.

1.4.3 Cyanide analysis and waste liquor characteristics

It is recommended that any future work is conducted based on a solid knowledge of the waste characteristics and not by the "black box" approach. Data are required particularly on pH variations, concentrations of cyanide (CN'), thiocyanate and metal cyanide complexes (especially ferrohexacyanide) in the liquors, ponds and waste streams, those locations for which treatments are to be developed.

2.0 INTRODUCTION

The cyanide present in industrial waste liquors has many chemical forms depending on the industrial process and factors such as pH, metallic and non-metallic content of the liquors.

To reduce the cyanide content, many chemical methods have been developed to achieve cyanide removal and/or destruction, including alkaline chlorination, ozonation, hydrogen peroxide oxidation, electrolytic regeneration and destruction, conversion to "less toxic" forms, e.g., CNS and $Fe(CN)_6$, and finally direct acidification-volatilization-reneutralization.

Physical-chemical techniques involve ion exchange, activated carbon adsorption and ion flotation. There is also a category of procedures which Ingles and Scott (1978) refer to as natural degradation or lagooning which includes the processes of volatilization, oxidation and biodegradation. Gradual loss of cyanide in waste holding ponds is brought about through the physical-chemical forces of photodecomposition by sunlight, acidification by atmospheric CO_2, oxidation by atmospheric O_2, and adsorption on solids. Furthermore, bacteria have been indicated as effective degradation agents.

Effluent holding ponds are frequently the habitat of a variety of micro-organisms and there is increasing evidence that significant microbial degradation of cyanide and CNS may be occurring in these ponds. Bacteria isolated from gold tailings reportedly oxidize CN^- at pH 10 with cyanide as the nitrogen (N) source for bacterial growth. CNS was preferentially utilized over CN^- (Scharer et al, 1984). CN^- is the N source for Pseudomonas fluorescens which oxidizes CN^- to NH3 (Harris & Knowles, 1983). Excellent reviews of cyanide metabolism in micro-organisms have been published (Knowles, 1976; Castric, 1981).

In a gold mine tailings pond (Dome Mines) the increase in bacterial counts during the summer was correlated with a dramatic decline in the CNS concentration, but evidence for degradation of CN^- was inconclusive (Schmidt et al, 1981). The beneficial role of cyanide-metabolizing bacteria was recognized by the Homestake Mining Company and they have an operational biodegradation component in their effluent processing system (Mudder & Whitlock, 1984).

Mudder and Whitlock (1984) state that "biological treatment was examined and evaluated on a pilot scale, yielding a simple cost-effective process, the performance of which equalled or exceeded that of all other processes evaluated, while producing an environmentally compatable effluent".

The key in the Homestake treatment -- and the major tenet in the Boojum concept of "passive polishing" -- is utilization of biota which are already thriving in the unique habitats of the waste streams. Those may adsorb, metabolize or otherwise

remove undesirable solutes from the waste liquors, hence polish the waste water passively, by their mere presence. Passive polishing by actively promoting an indigenous flora appears to be particularly suited for situations where effluent treatment is complicated by large variations in flows and pH's. The indigenous flora, if present, has developed under those conditions and is likely tolerant to such variations. To produce a consistent effluent quality, extreme conditions in the effluent stream are backed up by bioadsorbent barriers which can be utilized when required.

The bioadsorbent potential of both living and dead Chara, a green alga, was recently described and defined in caveat # 32695 of Boojum Research Ltd. (Appendix A). Chara has been suggested as bioadsorbent for radionuclides in uranium tailings effluents. Given the ability of Chara to bind cations, one would not be too surprised if CN^- or metalo-cyanide complexes with negative valences (-1 to -4) did not adsorb to Chara. However, Chara species are known to absorb preferentially Fe (Blake & Dubois, 1982). This characteristic indicated some potential use as a bioadsorbent for metal-cyanide complexes.

Blue-green algae, also called cyanobacteria, because in a strict botanical sense they are more closely "related" to bacteria than to algae, were likely candidates to grow in seepages from the red mud lakes and in waste streams at Arvida from the Vaudreville Works. Typical blue-green algae are encased in a mucilaginous sheath which harbours a bacterial flora (Carr & Whitton, 1982).

In order for algae and bacteria -- any organism -- to grow, they must be provided with a nitrogen source. Blue-green algae groups are often physiologically incapable of utilizing atmospheric N_2. NO_3^- is apparently very low or absent in the waste liquors. A nitrogen source does exist however, in the form of cyanide. Utilization for bacterial growth has been discussed earlier. Experiments with the blue-green alga, Nostoc muscorum, showed that cyanide and even low concentrations of azide (N=N) could serve as N sources for growth (Rai, 1978). Methylamine, with a chemical backbone similar to HCN, i.e. H_3CNH_3, was an organic N source for the blue-green, Anabaena doliolum (Rao & Singh, 1983). It is therefore reasonable to hypothesize that if "blue-green alga-bacteria assemblage" are present in the waste streams, cyanide could serve for these algae also as a nitrogen source.

3.0 MATERIALS & METHODS

3.1 'The Chara process'

3.1.1 Preliminary growth experiments

Chara globularis was collected from Guelph Pond, near Guelph, Ontario, and maintained in the laboratory in large aquaria containing Guelph Pond water. The light intensity was ca. 100 $uE.m^{2}.s^{-1}$ and the photoperiod was 12 h L: 12 h D. Material for the Chara growth experiment in Arvida waste effluents and cyanide adsorption experiments utilized the laboratory stock culture of Chara.

Ten, 3-4 cm long apical sections of Chara plants (4 whorls of lateral branches per section) (see Fig. 1) were placed in 1 litre jars containing water samples (A1 - A5) from Arvida as well as two Guelph Pond water samples (controls). Appendix B gives a more complete characterization of all Arvida samples, e.g., pH, conductivity, etc., as of their arrival at the laboratory of Boojum Research Ltd.

The health and growth of Chara was monitored at the following intervals: 18, 42, and 114 h; then 5, 6, 7, 8 and 10 days (see Figure 1).

3.1.2 Preliminary adsorption experiments.

3.1.2.1 Cyanides in pot lining leachate

Chara was air-dried for 4 days and then treated as described below. Some was oven-dried at 40°C for 24 hours to provide the Chara "natural" type. Some Chara was decalcified in 0.1 N HCl for 1.5 hours, then dried at 40°C for 24 hours to provide the Chara "decalcified" type. The "$FeCl_3$-treated" Chara was prepared by soaking the air-dried Chara in 400 mls of 1.1 M ($FeCl_3$) for 1.25 hours then rinsing in distilled water in a neutral pH and drying for 24 hours at 40°C. The "decalcified $FeCl_3$" treated Chara was prepared by first decalcifying the Chara, then treating with $FeCl_3$ as described previously, before drying at 40°C for 24 hours. Two replicates (3.0 grams each) of each preparation of Chara were used.

Only one Arvida test solution was employed: 'liquor #1 (pot-lining leachate) at its original pH of 11.9 (~pH 12). A 250 ml volume of liquor #1 was adjusted to pH 9. The final pH after the addition of 10 mls of 1.0 N HCl to 250 mls of liquor #1 was 8.9 (~pH 9).

Five 50 ml aliquots of each liquor (pH 9 and pH 12, respectively) were dispensed into 100 mls plastic snap-cap

Figure 1. Photographs of Chara globularis plants after 10 days of growth in the laboratory. Note: short lateral branches arise as whorls on the main axis of the plant.

(A-B) Plants growing in Guelph Pond water (controls); (C) plants growing in B-stream water and (D) C-stream water.

vials. One vial of each pH was kept as a control by receiving no further treatment. One vial of each pH was then treated by adding one of the Chara types. The vials were closed and placed in tray on the lab bench (temperature of 20°C; darkness) for 1 week.

At the end of the incubation period the vials containing the Chara were opened and filtered through a nitex mesh screen and into a beaker. The Chara was discarded and the filtered solution was decanted into the original plastic vials used for the experiment. The control vials for each pH were not opened and not filtered. All of the vials were wrapped and shipped to Alcan for cyanide analysis.

3.1.2.2 Uptake of ferro-^{14}C-cyanide in spiked pot lining leachate.

Two ml of the pot-lining leachate (pH 12 or titrated to pH 9 with 0.1 N HCl) were pipetted into 20 ml glass scintilation vials. The vials were spiked with 100 ul of $Na_4Fe(CN)_6^{-4}$ (sp. ac. 50-70 $mCi.nM^{-1}$) and allowed to equilibrate for 10 min. before adding 100 mg of the 4 kinds of Chara material described above. 100 mg of Whatman filter paper #1 (untreated or $FeCl_3$-soaked) served as controls.

Three replicates of each treatment were prepared. One replicate of each of the plant or filter treatments was sacrificed at time zero to give an initial value; the remaining 2 replicates were incubated for 4 hours in closed vials. Two replicates of each waste liquor were labelled and incubated without plants or filters for 4 hours as a blank treatment.

Aliquots of 50 ul solution were removed from each treatment vial at zero time and after intervals of 1, 2, 3 and 4 hours in order to follow the time course for the adsorption of labelled cyanide from the solutions. The 50 ul aliquots were added to Nalgene bags (3 ml volume). ACS scintillation cocktail (3 mls) was added to these samples and the Nalgene bags were sealed and placed in 20 ml glass scintillation vials.

At the end of the 4-hour incubation, the plant- or filter-containing samples were filtered through 0.45 um millipore membrane filters and rinsed with dH_2O. The Millipore filter plus the plant or filter material were placed in a Nalgene bag and processed as described for the solution. Blanks of the Millipore filters alone were checked to determine labelled cyanide adsorption. Background levels were checked using the unlabelled liquors; all values were below 60 cpm. The activity of the added label (100 ul) was determined (to be about 180,000 cpm). All samples were stored in the dark overnight before being counted on a Beckman LS-230 Liquid Scintillation Counter. Results are reported as counts per min **(cpm).**

3.2 Indigenous algae at Arvida.

3.2.1 Collection and identification

Appendix B gives the locations of the collections made on the Arvida premises. Collections were made on 10 July, 19 October, by Alcan personnel and on 26 October 1984, by M. Kalin with assistance from Alcan personnel and dominant algal taxa were identified using standard taxonomic references and methodologies.

3.2.2 Culture methods for algae

Small mixed samples of algae were placed into 3-4 different liquid media (Bold's Basal Medium (BBM), BBM + soil extract, Chu -10) and/or plated out on BBM-agar (2%) plates or Chu-10 agar plates. In other experiments, samples of the Phormidium-Oscillatoria complex (see Figure 2) were teased out of the heterogeneous samples and cultured separately on the media. The chemical compositions of the media are given in Appendix C.

The cultures were grown in diffuse sunlight entering a SE window of the Boojum Research laboratory; some cultures received supplemental irradiation from fluorescent lamps (ca. 100 $uE.m^{-2}.s^{-1}$; 12 hL; 12 hD photoperiod). The temperature was ca. 23°C.

3.2.3 Growth of the "Phormidium-Oscillatoria

In this experiment conducted in November 1984 to January 1985, growth of the Phormidium-Oscillatoria, isolated from the 10 July, 1984 collection (AB-1) was compared in 4 different liquid media in an experiment lasting 48 days. A "sediment extract" was prepared by steaming a sediment-distilled water decoction for 5 min. at 121°C and 15 psi, and then filtering the water. Aliquots were added as a nutrient supplement to various media to give a final concentration of 10% sediment extract. The sediment-mud-organic matter for this preparation came from the C-stream. The four defined culture media tested were: Bold's Basal Medium, Cyanophycean Medium, Allen's Medium, and Anacystis Medium adjusted to pH 8 or pH 10. Growth conditions (light and temperature) were described above. Growth was determined as a dry weight increase in algal biomass in 3 days; growth was assessed by comparison to the day one weights.

3.2.4 Preliminary experiment: CN as N source

In this experiment, conducted in January to February 1985, used the same isolate of Phormidium-Oscillatoria. Bold's Basal Medium (without sediment extract) was used as the experimental medium to which varying amounts of KCN (0, 0.5, 1.0, 2.5, 5.0

Figure 2. Photographs of a "Phormidium-Oscillatoria-bacteria assemblage" from the Arvida B-stream. (A-B) Filaments of Phormidium and Oscillatoria (A magnification, x625; B magnification, x140); (C) large-celled filament of Oscillatoria among small-celled filaments of Phormidium (magnification x1500); (D) filaments of Phormidium (magnification x1500).

mM) were added in the presence or absence of nitrate nitrogen (2.9 mM). The phosphate concentration of the medium was reduced by half to alleviate problems of precipitation at the high pH. EDTA and $FeSO_4$ were omitted to avoid problems with cyanide complexing. The pH was adjusted to 10 with 0.1 N NaOH. The medium was autoclaved for 20 min. at 121°C and 15 psi and allowed to cool before cyanide additions were made. At the conclusion of the experiment, the pH was measured. Other growth conditions were as described above. There were 3 replicates per treatment and dry weights were determined at day 14 and 35 and compared to day 0 values.

3.2.5 Preliminary experiment: HCN conversion to CO_2

This experiment (January 1985) utilized algae from the same stock which supplied algae for all the earlier experiments (AB-1). 100 ul $K^{14}CN$ (sp. ac. 2-10 $mCi.mM^{-1}$) were added to 5 dram glass vials containing 1 ml BBM ($-NO_3$) with 1 mM KCN and either: (1) 2 mg killed algae (preheated at 100°C for 5 min); (2) 2 mg living algae; or (3) blank (no algae). A small piece of filter paper soaked in phenethylamine was suspended inside the vials with string and after addition of the $K^{14}CN$, the vials were stoppered for a 24 hour incubation period. At the end of this time the vials were opened, and 50 ul of 1.0 N HCl were added. The vials were re-sealed and gently swirled for several minutes to facilitate the dissolution of $^{14}CO_2$ (and $H^{14}CN$!) and subsequent adsorption on the phenethylamine-soaked filter paper. The radioactivity on the filter papers was then analyzed by liquid scintillation spectroscopy.

3.3 Cyanide analyses and waste liquor characteristics.

3.3.1 General procedures---waste liquors.

Waste liquors were received during June and July of 1984 in 2 gallon PVC bottles. Upon receiving the waste water, pH and conductivity were immediately determined. The samples were stored in darkness in closed containers until ready for use in experiments. Boojum Research Ltd. had agreed to carry out the work under a non-analysis agreement, hence no waste liquors were analyzed. Materials related to the wastes were prepared for analysis and shipped to Arvida for processing. A "black box" approach was adapted for the preliminary investigations.

3.3.2 Cyanide in Phormidium-Oscillatoria cultures.

At the conclusion of the experiment where cyanide (KCN) was used as a nitrogen source for Phormidium-Oscillatoria growth, the pH of the media was measured and cyanide (total and "free") were analysed by the Ontario Research Foundation. These analyses were felt necessary to demonstrate the need for'

continuous cyanide measurements and to gain confirmation of cyanide chemistry in culture medium. As a check on the accuracy of their methodology, several samples were included which contained cyanide (KCN; pH 11.0) at 0, 0.5 and 5.0 mm (0, 12.5 and 125 ppm, respectively).

3.3.3 Cyanide(s) in sediment extracts

A 2 cm layer of sediment-organic matter from 7 sites in the B stream sites was placed on the bottom of a 250 ml Erlenmeyer flask and covered with 100 ml of distilled H_2O; these samples were steamed at 121°C for 5 min. in an autoclave. After cooling and settling of sediment, the extract was filtered and the pH was taken. The soil extracts were stored in darkness in a refrigerator (ca. 4°C). Finally, in February 1985 the soil extracts were sent to Alcan for analysis of total and "free" cyanide. Theoretically these samples should contain no cyanide after this treatment.

4.0 RESULTS AND DISCUSSION

4.1 The Chara process

4.1.1 The growth potential of Chara spp.

Chara spp. are ecologically adapted to living in alkaline environments (pH 7- > 9) where the calcium and sulfate concentrations may be as high as 130 mg/L and 225 mg/L, respectively, and some species inhabits salt springs (Hutchinson, 1975). Charophytes reportedly accumulate more iron than any other aquatic plant (Blake & Dubois, 1982); 0.9 to 1.4% dry wt. according to Baszynsky & Karczmarz (1977). The major site of heavy metal binding by Chara (e.g., Cu, Hg, Zn, Fe, Ag, Sr, Cd et al.) is the cell wall (Jahnke et al. 1981).

Thus the metal adsorption by Chara is a physical-chemical process occurring equally well on living or dead material. Taken together, the growth (habitat preference) and cell wall characteristics of Chara suggest that it could function as (1) a passive polishing agent growing in situ in appropriate mine effluents or (2) as an effective bioadsorbent for metal and/or metal o-complexes.

4.1.2 The survival of Chara globularis

Table 1 gives the results of the Chara growth experiment. In comparison with algal growth in the two control flasks containing Guelph Pond water, Chara survived for up to 8-10 days in the Arvida waste water samples. At present we do not know what factor(s) were most injurious to the health of the Chara growing in effluent streams B and C. As alkaline lakes (ca. pH 9) are a natural habitat of Chara, the high pH of the liquors may not be the major factor contributing to the death of the Chara, except in liquors 1 and 3 where the pH was >12.0.

Survival of Chara globularis, a species not adapted to saline habitats in the liquors suggests that Chara evaluta or Chara aspera populations from saline environments, i.e. growing in salt ponds around Swiftcurrent, Medicine Hat, or Vernon, B.C. may be more effective in the Na-laden liquors of Arvida. Unfortunately the saline (Na rich) characteristics of the waste liquors was only brought to our attention after the growth tests had been carried out with the available stock cultures of C. globularis. Saline species, growing in saturated solutions and relatively murkey waters may be best suited for passive polishing in the red mud ponds.

4.1.3 Preliminary adsorption experiments

Experiments were conducted in the face of reasonable doubt given the cation exchange characteristic of the Charophyceae.

TABLE 1

SURVIVAL OF CHARA GLOBULARIS IN 5 WASTE WATERS

pH[1] (initial) (final)	Time in culture: Hours 18	42	114	Days 5	6	7	8	10
POT LINING LEACHATE								
(12,6)[1] (10.2)	alive	limp	death start	dead				
(10.75)[2] (10.20)	alive	limp	death start	dead				
B-STREAM								
(6.6) (8.15)	alive	alive	slightly limp	no change	limp	limp, still growing		
(7.9 I (8.15)	alive	alive	alive	alive	slightly limp	no change	limp	
POND WATER								
(12.0) (12.0)	alive	limp	no change	death start	dead			
(11.95) (12.0)	alive	very limp	no change	death start	dead			
SCRUBBER SLURRY								
(7.2) (8.8)	alive	semi-alive	limp	death start	dead			
(8.0) (8.8)	alive	limp	very limp	death start	dead			
C-STREAM								
(9.2) (7.8)	alive	alive	alive	limp	limp	limp	death start	
(8.9) (7.8)	alive	alive	alive	semi-alive	semi-alive	limp	death start	

1 - 1st test
2 - 2nd test
Controls: Run in duplicate and all plants were alive at the termination of the experiment; pH of Control #1 was 7.8 and 8.3 and Control #2, 7.8 and 8.7 beginning and end respectively.

However, interesting and useful results were obtained which suggested that Chara may have a metalo-cyanide bioadsorbent capability.

4.1.3.1 Soaking Chara in pot lining leachate

Table 2 shows the concentrations (mg/L^{-1}) of total and free cyanide remaining in potlining leachate at the end of the experiments in which Chara was employed as a potential bioadsorbant. The initial concentration of total and free cyanide were not determined at the beginning of the experiment, but a reasonable measure of these values may be seen in the "control" values, i.e. vials without Chara, sealed at the beginning of the experiment and not opened until they reached Alcan for cyanide analyses.

However, the latter free cyanide value may be a slight underestimate of the initial concentration of CN^- present at the beginning of the experiment. Some air was likely contained above the solution and, therefore, shaking during transportation of the vial could have produced volatilization and oxidization of cyanide. At both pH 12 and 9, there was a dramatic reduction in total and free cyanide in the vials which contained the natural, decalcified and $FeCl_3$-treated Chara.

Simovic (1984) has recently determined the effect of UV light, temperature and metals on the loss of HCN from solution. There is a rapid loss of HCN even at pH 10 from non-aerated solutions and there is a strong tendency for HCN formation from CN^- which is only rate-limited by dissociation of the various cyano-metal complexes which may be present in solution.

Figure 3 shows the equilibrium between HCN and CN^- in solution as a function of pH. The effect of chemically manipulating waste liquor #1 from pH 12 down to pH 9 to start the experiment produced a 30% reduction in total cyanide and a 50% reduction in "free" cyanide, i.e. HCN, CN^- and simple cyano-metal complexes, cf. Simovic, 1984). The physical processes of opening of the vials, together with aeration during the filtering and decanting of the Chara containing solutions probably volatilized much of the free cyanide prior to shipping the samples to Alcan for analysis.

Evidence for this interpretation may be seen in Table 2 for the total and free cyanide values at pH 12, but three additional assumptions are required: (1) that the total and free CN for the control is reasonably accurate; (2) that the total CN for the 4 experimental cbnditions really represent the strongly complexed cyanide (presumably $Fe(CN)_6^{-4}$) in solution; and finally, (3) that preparation of the Chara treated samples volatilized the greater part of the "free" cyanide present.

In the case of the control cyanide values, total (267 ppm) minus free (160 ppm) = 107 ppm of tightly complexed cyanide

TABLE 2

CYANIDE(S) REMAINING AFTER SOAKING OF DEAD *CHARA*

Chara Treatment	Cyanide in mg/l pH 12		pH 9	
	Total	Free	Total	Free
Control (no *Chara*)	267	160	180	80
Natural[1]	93	1.5	8.3	3.7
Decalcified	87	4.1	105	50
Natural - $FeCl_3$ treated	87	1.9	67	20
Decalcified - $FeCl_3$ treated	65	6.3	52	45

1 - *Chara* with normal accumulation of encrusting $CaCO_3$

Figure 3. Graphs showing the percentages of HCN and CN^- in solution as a function of pH.

initially present at the outset of the experiment. If we take an average of the 4 total CN values for the experimental Chara treatments, we obtain 63 ppm which may be within experimental error of the 107 ppm calculated as initially present at pH 12 under the experimental conditions used.

In other words, we suggest that the so-called "total" cyanide in the 4 experimental Chara treatments may closely approximate the residual, strongly complexed cyanide with "free" cyanide at negligible levels. Note that the corresponding "free" cyanide values for the 4 experimental vials are very low (between 1.9 and 6.3 ppm). We further suggest that the physical process of opening the vials, filtering the solutions and aerating them may have caused the major loss of "free" cyanide (see Simovic, 1984) and not the fact that Chara was functioning as a bioadsorbant. These results strongly suggest that a tight experimental design with immediate cyanide analysis should be derived for further work.

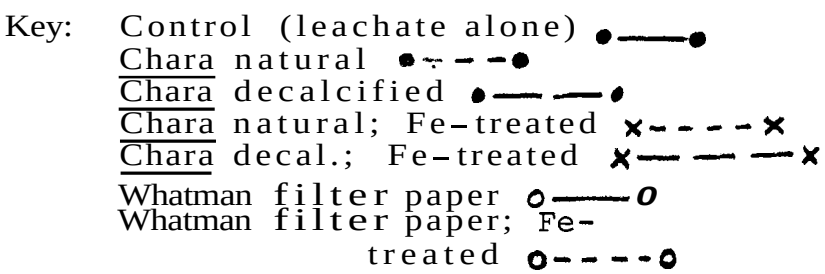
4.1.3.2 Fe-^{14}C-cyanide spiked pot lining leachate

The results of this experiment are shown in Figure 4. At pH 12, there was no significant adsorption of $Fe(^{14}CN)_6$ to any of the treated or untreated Chara or Whatman filter paper. At pH 9, however, there was reduction of $Fe(^{14}CN)_6^{4-}$ in solutions in which $FeCl_3$ treated Chara (both calcified and decalcified) was soaking. About 75% of the radioactivity was removed from the leachate within 4 hours.

Is there an apparent discrepancy between these results and those in 4.1.3.1 above, especially with regard to the pH 12 data? The inference from the results above at pH 12, is that strongly complexed cyanides such as $Fe(CN)_6^{4-}$ remained in solution in spite of the presence of the 4 kinds of Chara. The result from the tracer experiment with $Fe(^{14}CN)_6^{4-}$ at the pH 12 supports this interpretation as there was no removal of $Fe(^{14}CN)_6^{4-}$ from solution by Chara (Fig. 4) at that pH.

It is interesting that a considerable amount of tracer was removed from solution at pH 9. One possible explanation for the difference in the apparent capacity of Chara to bind $Fe(CN)_6^{4-}$ at pH 9 and not pH 12 may be due to a saturation of the Chara anion binding sites with OH^- at pH 12. It should be noted that as a bioadsorbant, Chara may be expected to exhibit an exchange capacity which varies as a function of pH (cf. Tsezos and co-workers studies on uranium binding to fungal hyphae of Rhizopus, e.g., Tsezos, 1984) and there is no a priori reason to expect otherwise.

Figure 4. Radioactivity (cpm) remaining in solution after soaking Chara and Whatman filter paper in pot lining leachate (pH 12 and 9) containing ferro-^{14}C-cyanide.



4.2 Indigenous algae at Arvida

4.2.1 Growth potential of algae-bacteria assemblages.

The Phormidium-Oscillatoria-bacteria assemblage was collected at numerous locations in the waste streams. (Table 3). Stream B and C have reportedly large variations in flows and pH's. Why are these blue-green algae growing there? These are highly adaptable organisms (along with their associated bacterial flora) which have the ability to colonize -- frequently via airborne vectors -- aquatic habitats which have highly variable physical and/or chemical environments, e.g. splash zones of marine and freshwater shorelines, hot springs, deserts, etc. (Carr & Whitton, 1982). Because of their dominance in the aquatic areas at Arvida, the Phormidium-Oscillatoria-bacteria assemblage have indeed "shown themselves" to be successful in coping with the variable but mostly high pH and the apparent lack of nitrogen other than CN^- (perhaps some NH_4^+). In a real sense these organisms are there because of and not in spite of the latter conditions.

4.2.2 Alaal identification

Table 3 lists the genera of algae identified in collections made at Arvida in 1984. The dominant taxon at each location is indicated. Of particular interest are the "macro" algae, as opposed to the unicellular algae such as Chlorella (sites AB 2-4). It is evident that the blue-green algae, particularly the genera Phormidium (tentatively identified as P. luridum) and Oscillatoria, were the dominant taxa at most of the alkaline sites.

Table 4 gives descriptions of stocks available from collections as cultures. Appendix B lists the locations where Phormidium-Oscillatoria were found with the same codes. At the least alkaline site. AB-6 with a pH of 6.8, the major algal taxon was the green filamentous'alga, Spirogyra sp. This algal complex has been found frequently on tailings sites. This group posesses also a mucaligenous sheet, which makes them suitable as a passive polishing agent. The "Phormidium-Oscillatoria -bacterial assemblage" grow as an inseparable mat covering the surface of sediments and rocks, as depicted in photographs of these genera (Figure 2).

4.2.3 Maintenance of algal cultures

Subsamples of the algal collection were cultured on a variety of liquid growth media and were also plated out on agar (Table 3). Stock cultures from the Arvida sites currently receive subsistence maintenance at Boojum Research Ltd. These stocks on subsistence are hoped to maintain the site-specific characteristics of the organism. If further work is required ,

TABLE 3

ALGAL IDENTIFICATIONS AND CULTURE STATUS

SITE NO.	ORIGINAL pH	DOMINANT ALGAL TAXA	CULTURE STATUS Liquid	Agar
AB-1	8.3	Oscillatoria Phormidium	AB-1 S.E.** BBM + AB-1 S.E. BBM Allen's Medium Anacystis Medium Cyanophycean Medium	AB-1 S.E. BBM
AB-2	10.8	Chlamydomonas Chlorella* (also yeast and bacterial colonies)	BBM BBM + AB-1 S.E.	Chu-10 BBM
AB-3	11.6	Chlorella*	BBM BBM+AB-1 S.E.	BBM
AB-4	10.3	Chlorella*	BBM BBM+AB-1 S.E.	BBM
AB-5	7.9	Anabaena(?)(small bluegreen filament) Oscillatoria* Chlamydomonas Chlorella* Spirogyra diatoms (Navicula?)	BBM BBM+AB-1 S.E.	Chu-10 AB-1 S.E. BBM
AB-6	6.8	Spirogyra* Ulothrix(?) (small green filament) Oscillatoria Phormidium	BBM BBM+AB-1 S.E.	Chu-10 AB-1 S.E. BBM
AB-7	10.0	Anabaena(?) (small bluegreen filament) Chlorella* Chlamydomonas (also ciliate protozoans/rotifers)	BBM BBM+AB-1 S.E.	BBM
AC-1	9.0	Oscillatoria Phormidium*	BBM BBM+AB-1 S.E.	BBM AB-1 S.E. Chu-10
ARM-1A	11.98	Small bluegreen filaments (growing slowly)		AB-1 S.E.
ARM-4A	11.3	Chlorella* Oscillatoria Phormidium* (also yeasts mold colonies and bacteria)	BBM+AB-1 S.E.	AB-1 S.E.

TABLE 3 (continued)

SITE NO.	ORIGINAL pH	DOMINANT ALGAL TAXA	CULTURE STATUS Liquid	Aaar
ARM-4B	10.7	Small bluegreen colonies (not identified)		AB-1 S.E.
ARM-41A	11.74	Oscillatoria* Phormidium*		AB-1 S.E.
ARM-41B	11.75	Oscillatoria Phormidium* Chlorella*		AB-1 S.E.
ARM-5	12.3	Oscillatoria Phormidium* Chlorella* Chlamydomonas (also yeasts, molds, bacteria)	BBM+AB-1 S.E.	AB-1 S.E.
ARM-6A	9.42	Phormidium* Oscillatoria	BBM+AB-1 S.E.	AB-1 S.E.
ARM-6B	10.1	Phormidium* Oscillatoria		AB-1 S.E.
ARM-7	11.98	Phormidium* Oscillatoria Chlorella Scenedesmus(?) diatoms (Navicula) (also bacteria, yeasts)	BBM+AB-1 S.E.	AB-1 S.E.
ARM-8A	10.8	Oscillatoria Phormidium* Phormidium(?) small bluegreen filament (unidentified) Chlorella* palmelloid green alga (unidentified)* (also yeasts, bacteria)	BBM+AB-1 S.E.	AB-1 S.E.
ARM-8B	11.4	Phormidium* Oscillatoria		AB-1 S.E.

* dominant taxon

** S.E. soil extract prepared from sediments collected from field sites

TABLE 4

SOURCES OF PHORMIDIUM STOCK CULTURES

SITE	pH	PRESENCE	GROWING IN
AB-1 (10/7/84)	8.3	dominant	AB-1 S.E. BBM+AB-1 S. E. BBM Allens Medium Anacystis Medium Cyanophycean Medium
AB-6 (19/10/84)	6.8	present but not dominant	AB-1 S.E. Chu-10 BBM BBM-AB-1 S. E.
AC-1 (19/10/84)	9.0	dominant	AB-1 S.E. Chu-10 BBM BBM+AB-1 S. E.
ARM-4A (26/10/84)	11.3	dominant	AB-1 S.E. BBM+AB-1 S. E.
ARM-41A (26/10/84)	11.74	dominant	AB-1 S.E.
ARM-41B (26/10/84)	11.75	dominant	AB-1 S.E.
ARM-5 (26/10/84)	12.3	dominant	AB-1 S.E. BBM+AB-1 S. E.
ARM-6A (26/10/84)	9.42	dominant	AB-1 S.E. BBM+AB-1 S. E.
ARM-6B (26/10/84)	10.1	dominant	AB-1 S.E.
ARM-7 (26/10/84)	11.98	common	AB-1 S.E. BBM+AB-1 S. E.
ARM-8A (26/10/84)	10.8	dominant	AB-1 S.E. BBM+AB-1 S. E.
ARM-8B (26/10/84)	11.4	dominant	AB-1 S.E.

A = Alcan B = B stream C = C stream ARM = Red mud pond

TABLE 5

SUMMARY OF ALGAL BIOMASS

TREATMENT	DAY	INITIAL CYANIDE CONCENTRATION (mM) 0	0.5	1.0	2.5	5.0
+ NO_3	0	1.63 + 0.21	1.63 + 0.21	1.63 + 0.21	1.63 + 0.21	1.63 + 0.21
	14	1.82 + 0.51	0.79 + 0.19	5.45 + 1.42	1.98 + 1.22	1.63 + 0.04
	35	1.80 + 0.42	1.23 + 0.41	1.25 + 0.33	1.95 + 0.22	1.52 + 0.44
- NO_3	0	1.63 + 0.21	1.63 + 0.21	1.63 + 0.21	1.63 + 0.21	1.63 + 0.21
	14	0.88 + 0.17	2.59 + 1.73	1.55 + 0.26	1.28 + 0.07	1.31 + 0.25
	35	1.79 + 0.18	1.18 + 0.12	1.05 + 0.22	1.47 + 0.50	1.52 + 0.24

Expressed as mg dry wt + 1 S.D. in Bold's Basal Medium supplemented with nitrate and/or cyanide as the nitrogen sources.

Legend: n = 2-3 samples

these collections will be used as sources for extensive culture production.

4.2.4 Growth of Phormidium-Oscillatoria

Particular emphasis was placed on culturing of the Phormidium-Oscillatoria collections on different media, at pH 8 and 10. One of the primary requirements for the development of a passive polishing agent is the ability to carry out experiments with the organisms to determine and manipulate their effectiveness.

The results of these experiments are shown in Figure 5. Best algal growth was achieved at pH 9 on Bold's Basal Medium with a "sediment extract" addition (10%). The blue-green algae did not grow on Allen's medium at pH 8. In general, growth was rather similar on 7 of the 9 media. In healthy cultures, the pH of the media is maintained or sometimes increased. In unhealthy cultures the pH decreases.

4.2.5 Preliminary experiments with cyanide

In order to facilitate strict comparisons among experiments and to produce some indication of general growth trends in a reasonable length of time, culture status was not emphasized and no additions were made to the original sample. The initial sample of Phormidium-Oscillatoria served as a source of algae for experiments conducted over a period of 7 months. However, by the time the second growth experiment was set up, the algae populations had apparently been exhausted.

4.2.5.1 Cyanide as a nitrogen source

In the growth experiment to determine whether the Phormidium-Oscillatoria-bacteria assemblage could utilize CN^- as a nitrogen source, the results are inconclusive (Table 5). Except for a spurious increase in algal weight at day 14 (NO_3 + 1.0 CN) none of the culture conditions displayed weight increases (compare with Fig. 5). Over the course of the experiment the pH decreased from about 10 to 6-7 at day 35, indicating that the cultures were not healthy.

4.2.5.2 Conversion of cyanide to carbon dioxide

The Phormidium-Oscillatoria-bacteria assemblage used in the preliminary experiment to determine whether $^{14}CN^-$ was removed from the culture medium, was taken from the same stock culture used for the growth experiment with CN^-. The results shown in Table 7 indicated that there was no significant difference between radioactivity trapped on the phenethylamine soaked filters in the blank, dead algae or live algae treatments.

Figure 5. Growth of the "Phormidium-Oscillatoria-bacteria assemblage" in various culture media. See Appendix C for culture media formulations.

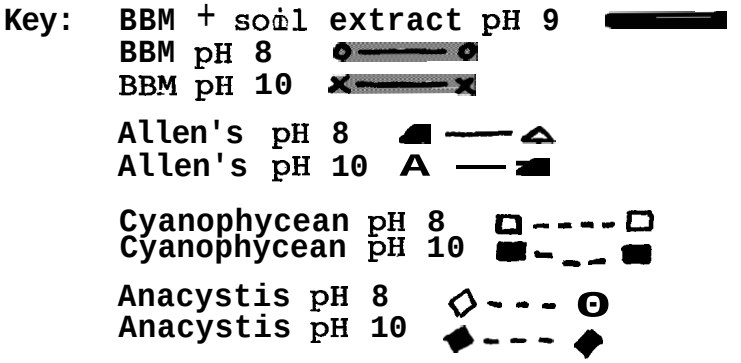


TABLE 6

CONVERSION OF CN TO CO_2

Trials	Radioactivity in cpm* Individual filter papers	mean ± 50
Control vial (without algae)	83, 146, 57	95.3 ± 45.8
Dead algae	82, 97, 102, 91, 168 67, 198, 80, 86	107.9 ± 44.4
Living algae	79, 187, 123, 322, 130,	135.9 ± 84.5

* counts per minute

The Phormidium-Oscillatoria experiment was also carried out with algae growing in the original sample, and it is reasonable to assume that cyanide was depleted in the solution. The sudden change to the new substrate with cyanide in the experiment did not allow the algae nor the bacteria to "gear up" metabolically for this new nitrogen source.

When switching from one nitrogen source to another, the alga must adapt internally, i.e. biochemically, to the change in substrate. These adaptations have been best studied in bacteria. For instance, when Pseudomonas fluorescens, was confronted with cyanide, one or more new proteins were synthesized, de novo, presumably in order to metabolize the new substrate (Harris & Knowles, 1983). Such biochemical changeovers are not immediate; there is a lag period before the new substrate can be efficiently metabolized.

So, apart from the technical difficulties inherent in our attempt to preferentially volatilize $^{14}CO_2$ and not $H^{14}CN$ from solution at the end of the ^{14}CN-feeding experiment, the fundamental design of the experiment has to be drastically modified. Future experiments have to fulfill the following conditions.

The algae have to be grown on CN as N source. The pH of the culture has to be maintained above 9 and monitoring of CN concentration during the growth and the experiments has to be carried out. Finally, and most importantly, the labelled carbon is to be traced in the algae and not in solution.

4.3 Cyanide analyses

4.3.1 Cyanide in Phormidium-Oscillatoria cultures

Appendix D shows the cyanide analyses performed on the culture filtrates at the end of the preliminary cyanide nutrition experiment. As the final pH of the cultures was 6.5 - 7.0, we anticipated results showing undetectable cyanide levels. ORF analyses confirmed our expectations. They also demonstrated analytical reliability in cyanide determinations by correctly evaluating standards which were interspersed with the experimental culture samples (Appendix D).

4.3.2 Cyanide in sediment extracts

Figure 6 shows the general trend of pH during storage of the various waste liquors from the C and B stream particularly. Although the containers are closed and stored in darkness at room temperature, the pH changes, and associated a corresponding shift from CN^- to HCN and when the bottles are opened, HCN escapes. It is obvious that "free" cyanide determinations after prolonged storage of water samples do not accurately reflect the cyanide levels present at the time of collection of the samples. Under ideal circumstances, any cyanide studies should necessitate the performance of cyanide analyses immediately prior to the experiment, especially in samples where the cyanide level is unknown. Preservation of samples for experiments with indigenous biota is inappropriate as the waste liquor would be altered by the addition of NaOH.

It can be concluded that red mud and potlining leachate are stable waste liquors with respect to pH whereas B and C streams and scrubber slurry have to be monitored constantly.

Table 7 shows the range of total and free cyanide found in the soil extract decoctions prepared from sediments consisting mainly of precipitates from the C stream. Perhaps the most important point is that these are presumably cyanide(s) which were released upon steaming the sediments during the preparation of the aqueous soil extracts. The extract prepared from sample AB-1 (see Appendix B) was used in the Phormidium-Oscillatoria growth experiment (see Fig. 5).

Practically speaking, based on theoretical considerations of cyanide, free cyanide should be non-detectable in these samples. This was the case in 9 liquors used to grow Phormidium-Oscillatoria mats in the laboratory. Free cyanide was consistentfy at <0.02 mg/l. Total cvanide concentrations ranged between 0.5 to <0.02 mg/l.

Figure 6. pH changes in waste liquors during storage in stoppered bottles and in darkness.

Source of samples: pot lining leachate (●——●); B-stream (○——○); red mud pond (■——■); scrubber slurry (□——□); C-stream (▲——▲).

TABLE 7

CYANIDE IN SEDIMENT EXTRACTS

Location	pH	Cyanide (ppm) Total	Free
AB-1[1]	8.0	2.87	0.17
AB-2[2]	6.8	2.07	0.03
AB-3	8.1	3.93	0.28
AB-4	7.4	0.025	<0.02
AB-5	7.8	13.7	0.64
AB-6	7.0	3.6	0.56
AB-7	7.3	0.91	0.02
AC-1	8.3	0.91	0.24

1 - soil extract prepared from sediment collected in July 1984.
2 - AB-2 through AC-1 = Enchantillon #1-7 collected in October 1984.

The analysis of the sediment extracts (Table 7) contained, however, some free cyanide. To make matters more complex, all samples were collected around two small ponds on the headwaters of the B stream. The variations in total and free cyanide concentrations are large in these sediment extracts. It is common to industrial waste streams to encounter variation, however, it emphasizes that the development of a successful treatment system for cyanide can only be carried out meaningfully in conjunction with the study of waste-cyanide chemistry.

It is essential that cyanide determinations be made by the researchers studying the question(s) at hand if meaningful quantitative value is to be placed on the cyanide data. It follows that a non-analysis agreement is unsatisfactory for effective research work.

5.0 CONCLUSION

From this feasibility investigation the most important conclusion is, that indigenous mat-forming algal-bacterial assemblages are abundant in waste streams at Arvida. Their cyanide tolerance is evident and the degradation ability of these biota of cyanide in the absence of any nitrogen source in the waste liquors can be inferred.

The experiments carried out during the feasibility study were generally weak, partly because of time restraints in addition to the concept chosen, namely a first shot "black box" approach. Hence the results were prone to "hindsight" understanding which is inherant in such an approach. This certainly applies to the behaviour of the biota and the waste liquors.

It is therefore concluded that bioadsorption and biodegradation of cyanide, particularly for waste water treatment have to be studied in conjuction with cyanide chemistry of the liquors to be treated to produce cost effective and reliable results.

In summary the feasibility investigation strongly suggests, that both the "Chara process" and the indigenous Phormidium-Ocilliatoria mats exhibited characteristics which could potentially serve as an effective passive polishing system. However before any firm potential can be assessed, a carefully planned, stepwise programm has to be carried out to arrive at definitive answers on the cyanide degradation ability of the indigenous biota and the adsorption capacity of metallo-cyanides on dead and growing Chara spp.

6.0 REFERENCES

Baszynsky, T. and K. Karczaarz. 1977. Investigations on the production of inorganic matter by Charophyta associations. I. Freshwater associations. Acta Hydrobiol. 19: 1-7.

Blake, G. and J.P. Dubois. 1982. L'epuration des eaux par les plantes aquatiques.. Etude de synthese sous contrat au Ministere de 1'Environnement. France. 103 pp.

Carr, N.G. and B.A. Whitton. 1982. The biology of cyanobacteria. Univ. Calif. Press. Berkeley and Los Angeles. xi + 688 pp.

Castric, P,A. 1981. The metabolism of cyanide by bacteria. In (B. Vennesland, E.E. Conn, C.J. Knowles and F. Wissing, eds). Cyanide in Biology. P. 232-261. Acad. Press. London-New York- Toronto-Sydney-San Francisco.

Harris, R.E. and C.J. Knowles. 1983. The conversion of cyanide to ammonia by extracts of a strain of Pseudominas fluorescens that utilizes cyanide as a source of nitrogen for growth. FEMS Microbiol. Letts. 20: 337-341.

Ingles, J.C. and J.S. Scott. 1981. Overview of cyanide treatment methods. (For presentation at: Cyanide in Grid Mining Seminar, Jan. 22, 1981, Ottawa, Ont.).

Knowles, C. J. 1976. Microorganisms and cyanide. Bacteriological Reviews 40: 652-680.

Mudder, T.I. and J.L. Whitlock. 1984. Biological treatment of cyanidation wastewaters. Soc. of Mining Engineers of AIME, Annual Meeting, Los Angeles, Calif. Feb-Mar 1984. Abs. R84-37, 9 pp.

Rai, R. 1978. Effect of azide and cyanide on growth and nitrogen fraction by the blue-green alga Nostoc calcicola Breb. In (Singh, S.P., D.N. Tiwari, A.K. Kashyap and P.K. Yadava, eds). Advances in cyanophyte research. pg 25-28.

Rao, J.P. and H.N. Singh. 1983. Methylanine regulation of heterocyst and nitrogenase in a methylamine resistant blue-green alga Anabaena doliolum. Zeitschrift fur Pf1anzenphysiol. 109: 69-80.

Scharer, J.M., J.J. Byerley, A.M. Charles and M. Moo Young. 1984. Outline of biohydrosetalurgy research (Appendix 12). Second Meeting of the Mineral Leaching and Metal Recovery Network (Bioninet) of the National Biotechnology Networks. Quebec City, P.Q. Aug 23, 1984.

Schmitt, J.W., L. Simovic and E. Shannon. 1981. Natural degradation of cyanides in gold. A progress report. Mining effluents. (For presentation at: Cyanide and the Gold Mining Industry Sesinar, Jan 22, 1981), pp 42.

Sinovic, L. 1984. Kinetics of natural degradation of cyanide from gold mill effluents. M.Sc. thesis, MacMaster Univ., Hamilton, Ont. XII, 369 pp.

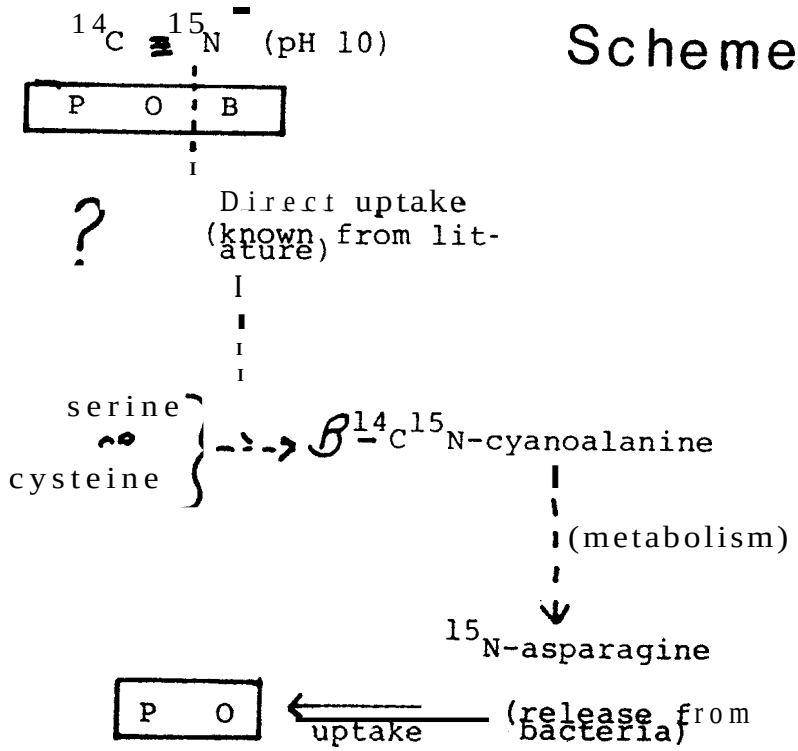
Tsezos, M. 1984. Recovery of uranium from bioloqical adsorbents ---desorption equilibrium. Biotechnology and- Bioengineering 26: 973-981.

Figure 6: pH changes in waste liquors during storage in stoppered bottles and in darkness.

Source of samples: potlining leachate ( ); B-stream ( ); red mud pond ( ); scrubber slurry ( ); C-stream ( ).

7.0 Appendices

Potential pathways of cyanide removal by Phormidium-Oscillatoria-bacteria (P-O-B)



Scheme B

asparagine can be used as a N source for bluegreen algae (Vaishampayan, 1982)